Insecticidal Activity of Indanomycin

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More than half of the worldwide expenditure on agrochemicals was devoted to insecticides in an effort to combat over half a million different herbivorous insect species[1]. Despite this, 15% of the crops planted are lost to feeding insects and other pests[2]. The cost of this damage and its effect on agriculture has increased the demand for more effective crop protection agents. For many years, pest control has been based primarily on the use of synthetic compounds[3]. Therefore, new insecticidal compounds with less environmental impact are sought to manage insects. Natural products are considered to be an ideal source for these requirements and several natural products have been developed as insecticidal agents[3].

The preliminary bioassays of extracts from a variety of nitrogen-fixing *Streptomyces* spp. obtained from China shown that one of the strains, *S. griseofuscus* (MS/ZD/033), produced the most active metabolite against mosquito larvae *Aedes aegypti*. Therefore, mosquitocidal-assay-directed purification of the cell extract from this strain afforded an insecticidal compound **1**.

Experimental

General

The NMR spectra in DMSO solution were recorded at 45°C on Varian VXR500 MHz spectrometer at Max T. Rogers NMR facility in the Department of Chemistry at Michigan State University. The UV spectrum in MeOH was recorded on a Shimadzu UV-260 spectrophotometer. CIMS and FABMS were obtained on JEOL JMS-AX505 and JEOL JMS-HX110 mass spectrometers at the Michigan State University Mass Spectroscopy facility in the Department of Biochemistry, which is supported in part by a grant (DRR-00480) from the Biotechnology Research Technology Program, National Center for Research Resources, National Institutes of Health. Circular Dichroism of compound **1** in MeOH was conducted on a JASCO J-710 spectropolarimeter (JASCO Incorporated, Japan). The nitrogen gas (99.99%) for CD analysis was generated by a nitrogen gas generator model NG-150 (Peak Scientific) at a flow rate of 20 L·minute^{-1}. The melting point was recorded on a Thomas Model 40 micro hot-stage apparatus and was not corrected. Final purification of compound **1** was performed on a recycling preparative HPLC model LC-20 and connected with a fraction collector model AS-20 (Japan Analytical Industry, Japan). The columns used were Shodex GS 3-10 2F (Asahi Chemical Industry, Japan) and Jaigel-ODS S-343-15 (Japan Analytical Industry, Japan).

Fermentation or Growth Media

The media for storing the cultures were: (1.09 g L^{-1} KH_2PO_4, 0.44 g L^{-1} $KHPO_4$, 0.0014 g L^{-1} $MgSO_4 \cdot 7H_2O$, 0.34 g L^{-1} NaCl, 0.34 g L^{-1} $CaSO_4 \cdot 2H_2O$, 0.01 g L^{-1} $FeSO_4 \cdot 7H_2O$, 0.0027 g L^{-1} $Na_2MoO_4 \cdot 2H_2O$, 9.1 g L^{-1} sucrose, 34.2 g L^{-1} KOH, 18 g L^{-1} agar and 114 g L^{-1} H_3PO_4). The media used in the fermentation or growth of organisms was liquid YMG (yeast extract 4 g·L^{-1}, malt extract 10 g·L^{-1} and glucose 4 g·L^{-1}); solid YMG (yeast extract 4 g·L^{-1}, malt extract 10 g·L^{-1}, glucose 4 g·L^{-1} and agar 18 g·L^{-1}); A9 (peptone 4 g·L^{-1}, glucose 10 g·L^{-1} and molasses 20 g·L^{-1}); PDA (potato dextrose agar 39 g·L^{-1}) and Emmons (neopeptone 10 g·L^{-1}, glucose 20 g·L^{-1} and agar 18 g·L^{-1}).

Artificial Diet for Insecticidal Assays

Gypsy moth eggs were obtained from The Forest Pest Management Institute, Sault Ste. Marie, Ontario, Canada (courtesy of Dr. B. MCCRON). Corn earworm and tobacco hornworm eggs were purchased from the insect rearing facility in the Department of Entomology, North Carolina State University, Raleigh, North Carolina.

The ingredients of dry diet for insects were: for gypsy moth; 36 g wheat, 7.5 g casein, 2.4 g Wesson's salt mix, 0.6 g sorbic acid, 0.3 g methylparaben (*p*-hydroxybenzoic acid methyl ester), 3.0 g Hoffman-LaRoche #26862 vitamin mix[4]; for corn earworm; 63.8% corn meal (gelatinized), 24.2% soy flour (defatted and toasted), 5% nonfat dry milk, 5% soy oil (refined and stabilized), 2% vitamin-mineral premix[5]; for tobacco hornworm; 100 g wheat germ (pre-ground), 45 g casein (purified), 40 g sucrose, 30 g torula yeast, 15 g salt mixture, 4 g ascorbic acid, 1.5 g sorbic acid, 1.0 g

methyl-P-hydroxy benzoate, 0.5 g cholesterol, 30 mg vitamins[6]. The insect diets were made as follows. The agar solution (1.8% agar for gypsy moth, 1.4% agar for corn earworm, 1.9% agar for tobacco hornworm) was held at 50°C and then added to the dry diet for gypsy moth (845 mg),[4] corn earworm (940 mg)[7] and tobacco hornworm (950 mg)[8] until the total diet weighed 5 g. Compound **1**, dissolved in 25 μl of DMSO, was mixed with the diet. Controls received 25 μl of DMSO alone. The diet was dispensed into 3.5 μl polystyrene vials (Sarstedt) and one larva was placed in each vial. Gypsy moth larvae were used at two to three days of age, corn earworm and tobacco hornworm larvae were neonates. Each treatment had fifteen replicates. The larvae were weighed at six days. Dunnet's test was used to determine the significance of weight reduction in these assays.

Mosquitocidal Assay

The mosquitocidal assay was conducted on the fourth instar mosquito larvae, *Aedes aegypti*, reared from eggs (Courtesy of Dr. ALEXANDER RAIKHEL, Department of Entomology, MSU) according to the published procedure[9].

Fermentation, Isolation and Purification of the Insecticidal Compound

Cultures of *S. griseofuscus* stored on inorganic medium were transferred onto YMG media slants and incubated for eight days at 26°C. These cultures then were transferred into 400-ml seed flasks containing 100 ml of YMG liquid medium. The inoculated flasks were kept on a rotary shaker at 110 rpm at 26°C for eight days, subcultured to 2-liter seed flasks containing 400-ml of A9 medium and were incubated on a rotary shaker at 110 rpm and 26°C for eight days. The fermentation broth of *S. griseofuscus* (6 liters) was centrifuged at 4°C and 10^4 rpm for 10 minutes to separate the mycelia from the broth. The wet cell-pellet (400 g) was extracted with MeOH : $CHCl_3$ (3 : 1, 800 ml) followed by 100% $CHCl_3$ (500 ml). The residual cell mass was discarded. The combined organic extracts were evaporated to dryness under the vacuum and yielded a powdered product (5.8 g). This product (5.8 g) was partitioned with MeOH : $CHCl_3$ (1 : 10, 15 ml × 4) and the soluble portion was evaporated to dryness (4.5 g). This product (4.5 g) was extracted further with acetonitrile (3 × 15 ml), and the soluble portion was evaporated to dryness to yield an amorphous powder (1.6 g). It was then fractionated on a GS 3-10 column using MeOH : H_2O (85 : 15) as the mobile phase at a flow rate of 5 ml·minute^{-1} and detected at 228 nm. The fraction with 42 minutes retention time (889 mg) was repeatedly purified by preparative HPLC on an ODS column using MeOH : H_2O (95 : 5) as the mobile phase at a flow rate of 3 ml·minute^{-1}. The peak at 76 minutes was collected and evaporated to dryness. The resulting product was a white crystalline powder, pure compound **1** (480 mg). The yield of compound **1** was 80 mg·L^{-1} of the fermentation broth.

Compound **1** melted at 98~101°C and gave an M^+ ion at *m/z* 493 in its FAB MS. This indicated $C_{31}H_{43}NO_4$ as the MF of the compound. Other major fragments observed in the MS were: 55 (15), 67 (18), 94 (100), 119 (18), 135 (15), 157 (14), 187 (10), 212 (10), 238 (10), 251 (65), 281 (9), 334 (6), 399 (7), 420 (5), 464 (80). It gave two UV λ_{max} (MeOH) at 243 (ε: 36630) and 289 nm (ε: 17847), respectively. Detailed 1D and 2D- ^{1}H- and ^{13}C NMR experiments along with the MS data revealed that compound **1** is identical to indanomycin[10~12].

Compound **1** was dissolved in MeOH (1 mg·ml^{-1}) and the CD was determined under the following conditions: scan mode: wavelength; scan range: 200~500 nm, sensitivity 500 mdeg, response 64 msec, scan speed 500 um·minute^{-1}, band width 1 nm, accumulation 0.2 nm·data^{-1}. Compound **1** exhibited an absorbance at 328.7 nm, and the CD value was $\Delta\varepsilon = -58.883$ mdeg.

Results and Discussion

Compound **1** was dissolved in ether and reacted with CH_2N_2 in ether. The resulting product gave a singlet at δ 3.65 for $COOCH_3$ in its ^{1}H NMR spectrum and confirmed the presence of a COOH group. Also, there was a broad peak at 9.58 ppm that exchanged with D_2O. Acetylation of this product with AC_2O in pyridine did not yield an acetate. This suggested that an amino group was present in compound **1** in addition to a –COOH group. ^{1}H NMR of the acetylated product did not show any change and confirmed the presence of a secondary NH group in **1**. The CD of compound **1** exhibited a strong negative absorption at 328.7 nm. The negative result showed that compound **1** absorbs more of the

Fig. 1. The structure of indanomycin, compound **1**.

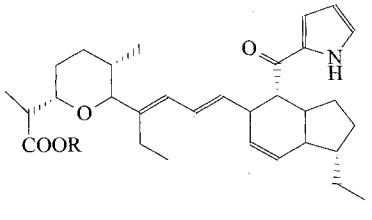
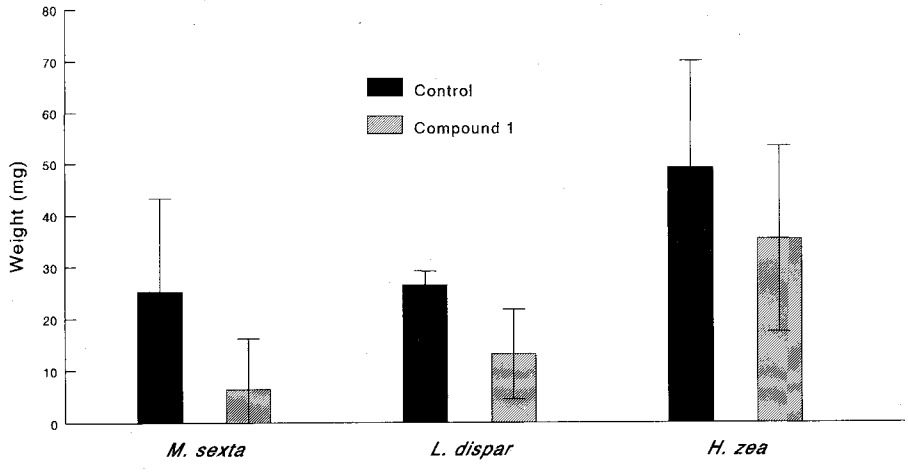


Fig. 2. Growth inhibitory assays of indanomycin against insects.



right-polarized light. A literature search on compound **1** based on its NMR spectral data confirmed that it is identical to indanomycin[10~12] as shown in Fig. 1.

In our tests, compound **1** showed bactericidal and insecticidal activities. It was active against *Streptococcus aureus* and *Staphylococcus epidermidis* at 1 ppm concentration, respectively. There was no activity against fungi, yeast or other test bacteria when tested at 100 ppm. Compound **1** gave significant weight reduction for tobacco hornworm (*Manduca sexta*) and gypsy moth (*Lymantria dispar*), but had only slight activity against corn earworm (*Helicovarpa zea*) in feeding trials using artificial diet. Compound **1** showed a 50% weight reduction for gypsy moth and tobacco hornworm neonate larvae at 100 ppm concentration at 6 days (Fig. 2.). Also, it reduced the weight of corn earworm at 100 ppm by 33% after six days (Fig. 2). Compound **1** gave 100% mortality on 4th instar mosquito larvae, *Aedes aegypti*, at 20 ppm. Indanomycin is reported to be an antibacterial antibiotic[10~12]. This is the first report of CD, insecticidal and mosquitocidal activities of indanomycin.

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References

1) LEY, S. V.; A. A. DENHOLM & A. WOOD: The chemistry of azadirachtin. Natural Product Reports. 10: 109~157, 1993
2) LEY, S. V. & P. L. TOOGOOD: Insect antifeedants. Chem. Bri. 26: 31~35, 1990
3) FABRE, B.; E. ARMAU, G. ETIENNE, F. LEGENDRE & G. TIRABY: A simple screening method for insecticidal substances from actinomycetes. J. Antibiotics 41: 212~219, 1988
4) BELL, R. A.; C. D. OWENS, M. SHAPIRO & J. R. TARDIF: Development of mass-rearing technology. The gypsy moth: research toward integrated pest management and development program. Forest Service and Education Agency, Animal and Plant Health Inspection Bulletin, 1584. USDA, Washington D.C. 599~655, 1981
5) BURTON, R. L.: A low-cost artificial diet for the corn earworm. J. Econ. Entomol. 63: 1969~1970, 1970
6) YAMAMOTO, R. T.: Mass rearing of the tobacco hornworm. II. Larval rearing and pupation. J. Econ. Entomol. 62: 1427~1431, 1969
7) JOYNER, K. & F. GOULD: Developmental consequences of cannibalism in *Helicoverpa zea* (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Amer. 78: 24~28, 1985
8) BELL, R. A. & F. G. JOACHIM: Technique for rearing laboratory colonies of tabacco hornworms and pink bollworms. Ann. Entomol. Soc. Amer. 69: 365~373, 1976
9) NAIR, M. G.; A. R. PUTAM, S. K. MISHRA, M. H. MULKS, W. H. TAFT, J. E. KELLER, J. R., MILLER, P.-P. ZHU, J. D. MEINHART & D. G. LYNN: Faeriefungin: a newbroad-spectrum antibiotic from *Streptomyces griseus* var. *autotrophicus*. J. Nat. Prod. 52: 797~809, 1989
10) BELOEIL, J. C.; M. A. DELSUC, J. Y. LALLEMAND, G. DAUPHIN & G. JEMINET: Application of the homonuclear and heteronuclear two-dimensional chemical-shift correlation NMR spectroscopy to the complete assignment of ^{1}H and ^{13}C NMR spectra of ionophorous antibiotic X. 14547 A. J. Org. Chem. 49: 1797~1800, 1984
11) LIU, C. M.; T. E. HERMANN; M. M. LIU, D. N. BULL, N. J. PALLERON, B. L. PROSSER, J. W. WESTLEY & P. A. MILLER: X-14547 A, a new ionophorous antibiotic produced by *Streptomyces antibioticus* NRRL 8167. Discovery, fermentation, biological properties and

taxonomy of the producing culture. J. Antibiotics 32: 95~99, 1979

12) WESTLEY, J. W.; R. H. EVANS, L. H. SELLO, N. TROUPE, C. M. LIU & J. BLOUNT: Isolation and characterization of antibiotic X-14547A, a novel monocarboxylic acid ionophore produced by *Streptomyces antibioticus* NRRL 8167. J. Antibiotics 32: 100~107, 1979